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Hepatoprotective activity of *Curcuma xanthorrhiza* Roxb. on paracetamol-induced liver damage in rats and correlation with their chemical compounds

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ABSTRACT

Background: Paracetamol (PCT) is a widely used drug, but at high dose, it can produce undesirable side effects such as hepatotoxicity. *Curcuma xanthorrhiza* Roxb. is the one of Indonesian medicinal plants that traditionally used to treat liver disorders. **Aim of the Study:** The present study was carried out to investigate the hepatoprotective activity of fresh rhizome decoction (FRD), dried rhizome decoction (DRD), essential oils, and curcumin of *C. xanthorrhiza* in Wistar albino rats. **Materials and Methods:** The liver toxicity was induced in rats using PCT. The blood samples and livers were collected and subjected to biochemical such as serum glutamic pyruvate transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT), gross macroscopical, and histopathological analysis. The chemical compounds and their hepatoprotective activity were correlated. **Results:** Among all samples, FRD showed better activity compared to the other samples with percentage protection of SGPT (99.45%) and SGOT (98.86%) level in a dose of 6.75 g/kg BW. **Conclusions:** From the results, it could be concluded that all the chemical compounds of C. *xanthorrhiza* have a hepatoprotective activity.

Keywords : *Curcuma xanthorrhiza* Roxb, curcumin, essential oils, hepatoprotective, paracetamol

INTRODUCTION

The liver is one of the largest organs in the body which plays metabolizing and detoxifying.[1] The liver is potentially susceptible to injury due to the exposure to drugs, environmental toxicants, and other xenobiotics.[2] One of the drugs that could potentially damage the liver is paracetamol (PCT). PCT is the most widely used analgesic and antipyretic.[3] High dose of PCT causes severe hepatic necrosis leading to acute liver failure.[4] PCT is metabolized by glucuronidation and sulfation by UDP-glucuronosyltransferases and sulfotransferases, respectively. The nontoxic glucuronide and sulfate metabolites are excreted in the urine. PCT can also be metabolized by hepatic cytochrome P450 enzymes to reactive metabolites, N-acetyl-P-benzoquinone imine (NAPQI).[5] NAPQI leading to the depletion of glutathione and covalent binds to proteins which leads to necrosis. The increase of these species can promote reactive oxygen and nitrogen species in hepatocytes, oxidative stress, mitochondrial permeability transition, and loss of ATP.[6] All these mechanisms are considered initiating events to cause necrotic cell death and implicated abnormalities of the liver.[7,8] In addition, the serum level of biochemical markers such as serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetic transaminase (SGOT), bilirubin, alkaline phosphatase, triglycerides, and cholesterol is elevated.[9]

A synthetic drug used to treat liver disorders often caused further liver damage, and therefore, the preference is being

shifted to herbal medicine.[10,11] Herbal medicines are known to play an important role in the treatment of various diseases, including liver disorders worldwide due to the high abundance, long-lasting curative effects, and few adverse effects.[12] This has led to increasing investigations into bioactive substances for liver disorders, which supposed to be the basis of bioactivities and health benefits through strong antioxidant and free radical scavenging abilities as well as anti-inflammatory action.[13] *Curcuma xanthorrhiza* Roxb. rhizome is one of the numerous herbal medicines with inherent antioxidant activity and has been used empirically in the management of liver disorders in communities.[14,15] *C. xanthorrhiza*, commonly called Javanese turmeric, belongs to the family Zingiberaceae.[16] It is a native plant in tropical zones of India and Southeast Asia, cultivated for culinary and medicinal purposes for many centuries.[17]

Conventionally, the rhizome is used for gastrointestinal disorders, liver disorders, nausea, and anti-inflammatory.[18] *C. xanthorrhiza* has also shown antioxidant, antidiuretic, anticancer, antihypertensive, antihepatotoxic, antibacterial, and antifungal effects.[19] Many phytochemical studies have been done on *C. xanthorrhiza* such as curcuminoids and essential oils as the major compounds and xanthorrhizol as a marker compound.[20] Despite its wide usage in traditional medicine, there is dearth information in the literature validating in the treatment of hepatoprotective activity and their chemical compounds. This study investigates the hepatoprotective properties of *C. xanthorrhiza* rhizome toward validating its ethnomedicinal usage.

MATERIALS AND METHODS

Materials and Chemicals

PCT (Konimex Pharmaceutical Industry), CMC-Na (Daichi), NaCl 0.9% w/v, formalin 10%, HE staining, SGPT kit (Dyasis), SGOT kit (Dyasis), natrium sulfate anhydrate, silica gel 60 plates (Merck), vanillin-sulfate acid reagent, fast blue salt reagent, ammonia vapor, citroboric reagent, and all solvents with analytical grade were used.

Plant Material

The rhizome of *C. xanthorrhiza* Roxb. was collected from Mangunan, Yogyakarta, Indonesia, in September 2016. The rhizome was identified by a botanist at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia, under the registration number UGM FA/4164/M/03/02.

Extraction and Isolation

Preparation of fresh rhizome decoction (FRD)

The samples were sliced of 4–6 mm thickness. The fresh slice of *C. xanthorrhiza* was extracted with boiling water at 90°C until a concentration of 50% v/w. This method as used empirically in the communities.[15] The FRD was kept at room temperature, and it was used for phytochemical studies, analysis of the essential oils contents, and *in vivo* studies.

Preparation of dried rhizome decoction (DRD)

The samples were sliced of 4–6 mm thickness and dried at 50°C in an oven for 3–4 days. The dried slice of *C. xanthorrhiza* was extracted with boiling water at 90°C until a concentration of 50% v/w. This method was used empirically in the communities.[15] The DRD was kept at room temperature, and it was used for phytochemical studies, analysis of the essential oils contents, and *in vivo* studies.

Preparation of essential oils from fresh rhizomes

About 6 kg of fresh rhizomes were sliced of 4-6 mm thickness and then were submitted to steam and water distillation apparatus for 300 min (until no more essential oils were obtained). The essential oils were collected, dried under anhydrous sodium sulfate, and stored at 4°C until analyzed. The yield of the essential oils (%v/w) is calculated as 100 × (volume of essential oils collected (mL)/100 g plant mass (g)). The essential oils from fresh rhizomes were used for *in vivo* studies.

Isolation of curcumin

One kg of *C. xanthorrhiza* powder was macerated with 10 L of ethyl acetate during 24 h with 10 min of stirring every hour for the first 6 h of maceration. After filtration, the residual material was remacerated by the same method with 5 L of ethyl acetate. The macerates were evaporated to obtained extract. Several portions of *n*-hexane were added and stirred to remove resins and essential oils. The curcuminoid fraction was then chromatographed on a silica gel column with chloroform as mobile phase. The first fraction was discarded, and the second fraction was collected and was checked by thin-layer chromatography (TLC) on silica gel F_{254} with chloroform:methanol (95:5 v/v) as mobile phase. The obtained curcumin was then dissolved in methanol and was recrystallized by addition of *n*-hexane. The isolated curcumin was analyzed using a Shimadzu TLC scanner and high-performance liquid chromatography for qualitative and quantitative analysis and *in vivo* studies.

Phytochemical studies

FRD and DRD were fractioned with *n*-hexane. The soluble fraction of *n*-hexane was collected and then evaporated for qualitative TLC analysis of essential oils profile and xanthorrhizol profile. Insoluble fraction of *n*-hexane was collected and then fractioned with ethyl acetate. The soluble fraction of ethyl acetate was collected and then evaporated for quantitative TLC-densitometry analysis of curcumin level. Insoluble fraction of ethyl acetate was collected and evaporated for qualitative TLC analysis of flavonoids profile.

Qualitative analysis of essential oils, xanthorrhizol, and flavonoids profile was performed using TLC on silica gel 60 plates. Essential oil profile was tested with toluene:ethyl acetate (93:7 v/v) as mobile phase. The spots were then observed under UV_{254} and then were treated by vanillin-sulfate acid spraying. The spots were heated at a temperature of 110°C for 10 min and observed under visible light. Xanthorrhizol profile was tested with toluene:ethyl acetate (93:7 v/v) as mobile phase. The spots were then treated by fast blue salt spraying and observed under visible light. Flavonoid profile was tested with tertiary buthanol:glacial acetic acid:water (3:1:1 v/v) as mobile phase. The spots were then treated by ammonia vapor followed by citroboric spraying. The spots were heated at a temperature of 110°C for 10 min and

observed under UV_{366} and visible light. Quantitative analysis of curcumin level was performed using TLC-densitometry on silica gel 60 plates and chloroform:methanol:glacial acetic acid (95:5:0.5 v/v) as mobile phase. The spots were then observed under UV_{366}. Quantitative analysis was performed by measuring the spot areas using the TLC scanner at λ_{max} 425 nm.

Analysis of the essential oils from FRD and DRD

FRD and DRD were analyzed quantitatively using Stahl distillation method and identification of the chemical compounds using gas chromatography-mass spectrometry (GC-MS) method. Quantitative analysis of essential oils level from FRD and DRD was performed using Stahl distillation. FRD and DRD were submitted to hydrodistillation with a Stahl apparatus for 120 min until no more essential oils were obtained. The essential oils were collected, stored and then were analysed by GC-MS. The yield of the essential oils (%v/w) is calculated as 100 × (volume of essential oils collected (mL)/100 g plant mass (g)).[20]

GC-MS analysis was used for the identification of the chemical compounds of essential oils from FRD and DRD. The chemical compounds were separated using a Shimadzu GCMS-QP2010S and equipped with a HP-5 capillary column (30 m × 0.25 mm). Samples were dissolved in *n*-hexane and were injected automatically in split mode (autoinjector Shimadzu) using pressure-controlled helium as a carrier gas at a linear velocity of 26.3 cm^3/min. The temperature of the injector was maintained at 300°C. The oven temperature was programmed from 60°C for 4 min, then at 5°C/min to 290°C, and held for 10 min. Samples were analyzed with EI electron impact mode with electron energy 70 eV; scan, *m/z* 28-600. Peak areas and retention times were measured by computerized integration. Chemical compounds were identified by comparing their mass spectral data with the existing Wiley library and National Institute of Standards and Technology.

In Vivo Experiments of Hepatoprotective Activity

Animal experiments

The healthy adult male Wistar rats (2–3 months old) weighing 150–300 g were used in the experiments. The rats were housed in polypropylene cages at 22±2°C with constant relative humidity of 55±10% and automatically controlled 12 h light and dark cycle (light on at 07:00 h). They were fed with standard laboratory animal feed and water *ad libitum*. The rats were analyzed SGPT and SGOT levels on day 0 at baseline to know the condition of the rats before the given of treatments. All performed procedures were approved by the Institutional Animal Ethical Committee, (number 02.02/04/UN1/LPPT/II/2017), Universitas Gadjah Mada, Indonesia.

Design of the *In Vivo* Experiment

Determination of dose of each group was based on empirical use of FRD in communities i.e., 25 g daily, and then was converted to dose for rats, i.e., 2.25 g/kg BW. In FRD group there was ranking dose, i.e., 1/3 of therapeutic dose (0.75 g/kg BW), therapeutic dose (2.25 g/kg BW), and 3 of therapeutic doses (6.75 g/kg BW). The dose of DRD was obtained from FRD which has undergone shrinkage weighting to 20%, and then, the dose was amount to 0.45 g/kg BW. In DRD group, there was ranking dose, i.e., therapeutic dose (0.45 g/kg BW), 3 of therapeutic doses (1.35 g/kg BW), and 9 of therapeutic doses (4.05 g/kg BW). The dose of essential oils and curcumin were obtained from the equivalent dose of FRD at 2.25 g/kg BW so the doses were 1.01 μL/kg BW and 75 μg/kg BW respectively.

The male Wistar rats were randomized into ten experimental groups with five animals in each group. The animals in Group I were served as vehicle control and orally received sodium carboxymethyl cellulose 0.5% for 9 days. Group II animals were served as induced control and orally received PCT (3 g/kg BW) for 3 days on the 7th, 8th, and 9th days. The animals in Groups III, IV, and V were served as test group and were treated orally with FRD of *C. xanthorrhiza* of 0.75 g, 2.25 g, and 6.75 g/kg BW for 9 days and orally received PCT (3 g/kg BW) for 3 days on the 7th, 8th, and 9th days. The animals in Groups VI, VII, and VIII were served as test group and were treated orally with DRD of *C. xanthorrhiza* of 0.45 g, 1.35 g, and 4.05 g/kg BW for 9 days and orally received PCT (3 g/kg BW) for 3 days on the 7th, 8th, and 9th days. The animals in Group IX were served as test group and were treated orally with essential oils of *C. xanthorrhiza* of 1.01 μl/kg BW for 9 days and orally received PCT (3 g/kg BW) for 3 days on the 7th, 8th, and 9th days. The animals in Group X were served as test group and were treated orally with curcumin of *C. xanthorrhiza* of 75 μg/kg BW for 9 days and orally received PCT (3 g/kg BW) for 3 days on the 7th, 8th, and 9th days. The bloods were collected on days 0 and 10 by means of capillary tubes through retro-orbital plexus. The blood samples were allowed samples to stand at room temperature for 30 min and then centrifuged at 4000 rpm for 10 min to separate the serum. The serum was removed for the assessment of biochemical parameters. On the 10th day, animals were sacrificed by cervical dislocation, and liver samples were dissected for the assessment of gross macroscopical and histopathological analysis.

Biochemical assay

Determination of SGPT was carried out using a GPT FS kit by DiaSys Diagnostic Systems GmbH. The principle of this test is to catalyze a reaction with pyruvate as one of the end products. Pyruvate reacts in the next reaction that involves oxidation of nicotinamide adenine dinucleotide hydrogen (NADH) to nicotinamide adenine dinucleotide. The rate of decrease of NADH was measured photometrically and was directly proportional to the rate of formation of pyruvate, which is indicative of SGPT activity.[21]

Determination of SGOT was carried out using a GOT FS kit by DiaSys Diagnostic Systems GmbH. SGOT is an enzyme that catalyzes the conversion of 2-oxoglutarate into oxaloacetate, which further reacts with NADH. The rate of decrease of NADH was measured photometrically and was directly proportional to the rate of formation of oxaloacetate, which in turn is indicative of SGOT activity.[21]

Gross macroscopical analysis

Liver samples of each group were observed if there were irregularities or abnormalities of color.[22]

Histopathological analysis

Small pieces of liver samples from each group were dissected and fixed in 10% formalin, dehydrated in graded ethanol (50–100%), and embedded in paraffin wax. Paraffin sections, which were 5-μm thick, were then stained for routine histopathological study using hematoxylin and eosin dye for microscopic observation of histopathological changes in the liver. Three animals were prepared for each group; each animal contains two sections. Liver sections were scored and evaluated by two observers of pathologist. Ten field areas for each section were selected and examined for histopathological changes under the light microscope. According to a Brunt system analysis with modifications, the liver fields were scored as follows: Normal appearance (−), mild cellular disruption in <25% of field area (+), moderate cellular disruption of 25–50% of field area (++), and severe cellular disruption of 51–100% of field area (+++).

Statistical Analysis

Data were analyzed using one-way ANOVA followed by *post hoc* LSD test using SPSS 18.00 for Windows software. The results are expressed as mean ± SEM. $P<0.05$ was considered as statistically significant between the treatment groups in this study.

RESULTS

Extraction and Isolation

The rhizome of *C. xanthorrhiza* was prepared in FRD, DRD, essential oils from fresh rhizomes, and isolated curcumin. FRD and DRD were yellow-brownish in color, smell, and taste a bit bitter. Essential oils from fresh rhizomes were brownish yellow in color, smell, and taste a bit bitter. Isolated curcumin was yellow in color, and the purity was 88.65%.

Phytochemical Studies

The phytochemical studies of FRD and DRD are presented in Table 1. TLC essential oil profile results under UV_{254} of FRD showed no spot and DRD showed five spots. Observations in visible light of FRD showed three spots with blue–purple and purple color and DRD showed six violet spots with blue-purple, purple, and red–purple color. The colors indicated that FRD and DRD contain essential oils.[23] TLC xanthorrhizol profile results under visible lights of FRD and DRD each showed one spot with orange–red color. The color indicated that the samples contain xanthorrhizol.[23] TLC curcuminoid profile results under UV_{366} of FRD and DRD each showed three spots with yellow color. The spots were curcumin and its derivatives. The results showed that the level of curcumin contained in FRD and DRD was 0.05% ± 0.01% w/w and 0.07% ± 0.01% w/w, respectively. TLC flavonoid profile results under UV_{366} of FRD showed three spots with yellow color and DRD showed two spots with blue color. The spots of FRD were predicted flavonol containing 3-OH free and have or do not have free 5-OH. The spots of DRD were predicted flavan and flavanone that do not contain 5-OH or flavonol without 5-OH free but substituted at 3-OH.[24]

Essential Oil Level and Their Chemical Compounds

Essential oil level and chemical compounds of FRD and DRD were investigated to correlate the compositions of essential oils and their hepatoprotective activity. Essential oil level of FRD and DRD was 0.05 ± 0.01% v/w and 0.04 ± 0.01% v/w, respectively. The list of chemical compounds is shown in Table 2. The chemical compounds of the essential oils have been previously reported in the present study, showing that

Table 1: Phytochemical studies of FRD and DRD

Chemical profile	FRD	DRD
Essential oils	+	+
Xanthorrhizol	+	+
Curcuminoids	+	+
Flavonoids	+	+

+: The sample contains chemical profile, -: The sample does not contain chemical profile. FRD: Fresh rhizome decoction, DRD: Dried rhizome decoction

Table 2: Chemical compounds of essential oils from FRD and DRD

Chemical compounds	RT	FRD (intensity)	DRD (intensity)
α-pinene	6.563	-	0.18
Camphene	7.064	0.33	0.35
Eucalyptol	9.466	-	0.12
Camphor	13.284	11.75	9.83
Terpinen-4-ol	13.381	0.78	0.62
α-terpinene	13.548	-	0.53
Borneol	13.649	0.31	0.31
δ-elemene	18.991	-	0.07
β-elemene	20.650	0.43	0.36
Zingiberene	21.018	0.59	0.44
α-bergamotene	21.149	-	0.12
Caryophyllene	21.294	-	0.16
γ-elemene	21.768	1.15	0.89
(E)-β-farnesene	22.385	1.17	0.86
Ar-curcumene	23.153	14.18	21.66
Furanodiene	23.510	4.72	3.67
α-cedrene	23.927	15.98	3.01
Germacrene B	25.058	0.90	0.72
Curlone	25.842	-	0.24
Curzerenone	26.276	10.93	10.90
β-bisabolol	27.742	-	1.00
Germacrone	28.447	6.11	6.25
Cis-β-elemenone	28.862	-	0.36
Xanthorrhizol	29.797	30.67	37.06
Isovalleral	30.657	-	0.29

RT: Retention time, FRD: Fresh rhizome decoction, DRD: Dried rhizome decoction

some compounds are differences and have a considerable variation in the levels of compounds. The variations may also be due to environmental factors such as the growth conditions and post-harvest processing.[14] There are 15 and 25 chemical compounds in FRD and DRD, respectively. Drying process often introduces artifacts compounds.[25] The five major compounds of FRD were xanthorrizol (30.67%), α-cedrene (15.98%), ar-curcumene (14.18%), camphor (11.75%), and curzerenone (10.93%). The five major compounds of DRD were xanthorrhizol (37.06%), ar-curcumene (21.66%), curzerenone (10.90%), camphor (9.83%), and germacrone (6.25%). Drying process affects the level and chemical compounds of essential oils.[26]

In Vivo Experiments of Hepatoprotective Activity

The effect of each sample on SGPT and SGOT levels in rats with induced PCT intoxication is shown in Tables 3 and 4, respectively. This study presented that SGPT and SGOT levels on day 0 were 30.5–107.3 U/L and 92.3–267.7 U/L, respectively, and there were no significant differences of SGPT and SGOT levels of each group.

The comparison between SGPT level of normal group with PCT group showed that PCT treatment increased SGPT level by 49.19 x ($P<0.05$). The SGPT levels of all treatment groups were then compared with PCT group. Statistical analysis indicated that the effects of FRD (0.75 g/kg BW), FRD (2.25 g/kg BW), FRD (6.75 g/kg BW), and essential oils decrease significantly the SGPT levels. The best protection effect (99.45%) was shown by FRD at the dose of 6.75 g/kg BW, meanwhile curcumin possessed only 49.23% of protection.

The comparison between SGOT level of normal group with PCT group showed that PCT treatment increased SGOT level by 14.32 x ($P<0.05$). The SGOT levels of all treatment groups were then compared with PCT group. Statistical

Table 3: SGPT levels in days 0 and 10

Treatment	Dose (orally)	SGPT (U/L)		% Protection
		Day 0	Day 10	
Normal	CMC-Na 0.5%	53.52±9.83	51.98±9.83	-
PCT	PCT 3 g/kg BW	59.48±5.11	2556.76±927.92#	-
FRD	0.75 g/kg BW	60.32±3.96	87.10±19.90*	98.60
FRD	2.25 g/kg BW	64.50±7.12	72.32±18.76*	99.19
FRD	6.75 g/kg BW	55.50±3.61	65.78±23.83*	99.45
DRD	0.45 g/kg BW	53.88±5.49	617.10±158.53	77.44
DRD	1.35 g/kg BW	62.18±11.85	521.86±240.59	81.24
DRD	4.05 g/kg BW	53.78±3.41	442.96±178.44	84.39
Essential oils	1.01 μL/kg BW	57.94±2.39	233.04±46.22*	92.77
Curcumin	75 μg/kg BW	72.42±4.83	1323.60±531.16	49.23

All groups were compared with PCT group. Values are mean±SEM, n=5 animals per group. All values are $P<0.05$ which was considered as statistically significant. (#) significant difference with normal group and (*) significant difference with PCT group. The percentage of the protection is calculated as 100 × (values of PCT-values of sample)/(values of PCT-values of normal control). FRD: Fresh rhizome decoction, DRD: Dried rhizome decoction, SGPT: Serum glutamic pyruvate transaminase, CMC: Carboxymethyl cellulose, SEM: Standard error of the mean, PCT: Paracetamol

Table 4: SGOT levels on days 0 and 10

Treatment	Dose (orally)	SGOT (U/L)		% Protection
		Day 0	Day 10	
Normal	CMC-Na 0.5%	171.06±14.17	141.42±24.81	-
PCT	PCT 3 g/kg BW	122.72±13.71	2025.62±409.18#	-
FRD	0.75 g/kg BW	163.14±26.72	306.70±51.98*	91.23
FRD	2.25 g/kg BW	152.78±8.31	196.78±19.20*	97.06
FRD	6.75 g/kg BW	136.82±4.02	162.86±27.34*	98.86
DRD	0.45 g/kg BW	163.96±13.50	745.48±145.61	89.37
DRD	1.35 g/kg BW	155.18±17.27	978.98±380.67	55.55
DRD	4.05 g/kg BW	129.52±8.16	408.20±123.34*	85.84
Essential oils	1.01 μL/kg BW	142.42±10.67	298.60±84.55*	91.66
Curcumin	75 μg/kg BW	184.16±8.19	1244.38±656.12	41.46

All groups were compared with PCT group. Values are mean±SEM, n=5 animals per group. All values are $P<0.05$ which was considered as statistically significant. (#) significant difference with normal group and (*) significant difference with PCT group. The percentage of the protection is calculated as 100 × (values of PCT-values of sample)/(values of PCT-values of normal control). FRD: Fresh rhizome decoction, DRD: Dried rhizome decoction, CMC: Carboxymethyl cellulose, SEM: Standard error of the mean, PCT: Paracetamol, SGOT: Serum glutamic oxaloacetic transaminase

analysis indicated that the effects of FRD (0.75 g/kg BW), FRD (2.25 g/kg BW), FRD (6.75 g/kg BW), DRD (4.05 g/kg BW), and essential oils decrease significantly the SGOT levels. The best protection effect (98.86%) was shown by FRD at dose of 6.75 g/kg BW meanwhile curcumin possessed only 41.46%.

Gross macroscopical study was performed on the liver to observe changes in the color of liver. Gross macroscopical of normal liver demonstrated dark maroon in color liver; meanwhile, intoxicated PCT liver showed major changes of the color from maroon to brown.[22] Groups of FRD (0.75 g/kg BW), FRD (2.25 g/kg BW), and FRD (6.75 g/kg BW) showed dark maroon in color liver. Groups of DRD (0.45 g/kg BW), DRD (1.35 g/kg BW), and DRD (4.05 g/kg BW) showed brown in color liver; meanwhile, treatment of essential oils and curcumin showed maroon to brown in color liver. The best effect was FRD (2.25 g/kg BW) group; meanwhile, the worst effect is DRD (0.45 g/kg BW) group. Histopathological analysis is important in determining the damage of liver and hepatoprotective effect of the treatment groups [Figure 1].

Histopathological study observed the structure and morphology of the liver and then demonstrated damage of the liver, such as inflammation, necrosis, congestion, hemorrhage, and steatosis.[22,27] In this study, normal group showed inflammation, congestion, haemorrhage and steatosis with mild cellular disruption and there was no necrosis. It means that the comparison between all treatment groups with PCT group must focus on level of cellular disruption and necrosis. The intoxicated PCT group showed inflammation, necrosis, congestion, and hemorrhage with moderate cellular disruption, and there was steatosis with mild cellular disruption. These pathological changes were found in all treatment groups. Inflammation with mild cellular disruption was observed in all groups of FRD, DRD (0.45 and 4.05 g/kg BW), and essential oils; meanwhile, moderate cellular disruption was observed on DRD (1.35 g/kg BW) and curcumin. Necrosis with mild cellular disruption was observed on FRD (2.25 g/kg BW), DRD (0.45 g/kg BW), essential oils, and curcumin; meanwhile, moderate cellular disruption was observed on FRD (0.75 and 6.75 g/kg BW) and DRD (1.35 and 4.05 g/kg BW). Congestion with mild cellular disruption was observed on FRD (2.25 g/kg BW), DRD (0.45 and 1.35 g/kg BW), and curcumin; meanwhile, moderate cellular disruption was observed on FRD (0.75 and 6.75 g/kg BW), DRD (4.05 g/kg BW), and essential oils. Hemorrhage with mild cellular disruption was observed on FRD (2.25 and 6.75 g/kg BW), all groups of DRD, essential oils, and curcumin; meanwhile, moderate cellular disruption was observed on FRD (0.75 g/kg BW). Steatosis with mild cellular disruption was observed in all groups of treatment. The best effect is FRD (2.25 g/kg BW) group; meanwhile, the worst effect is FRD (0.75 g/kg BW) group.

A comparison between all treatment groups against SGPT, SGOT, gross macroscopical, and histopathological analysis is possible for the difference of phytochemical studies of FRD and DRD such as differences and considerable variation in the levels of chemical compounds.

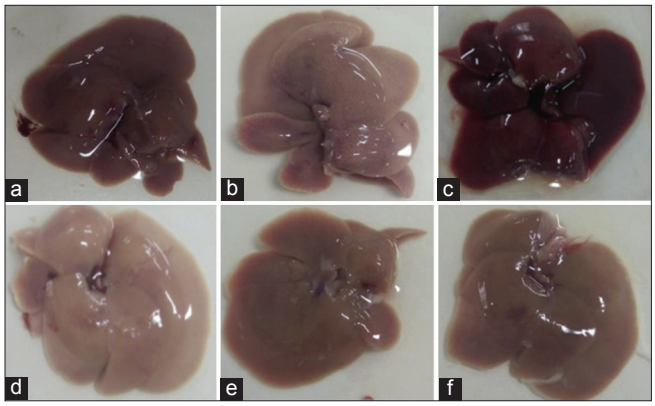

Figure 1: (a) Normal liver, (b) paracetamol (3 g/kg BW), (c) fresh rhizome decoction (2.25 g/kg BW), (d) dried rhizome decoction (0.45 g/kg BW), (e) essential oils, (f) curcumin

Correlation between Hepatoprotective Activity and the Chemical Compounds

The correlation between hepatoprotective effect especially for SGPT and SGOT levels with essential oils and curcumin contents of FRD and DRD can be performed using Pearson analysis. The result indicated that essential oils and curcumin contents of FRD and DRD had positive correlation with hepatoprotective effect on SGPT and SGOT levels [Table 5]. The effect of SGPT and SGOT protective has a positive correlation with the levels of essential oils and curcumin in the samples. These suggested that the essential oils had a stronger correlation against SGPT and SGOT parameter study than curcumin. The essential oils and curcumin, at least partly, contributed to the hepatoprotective activity and the others of chemical compounds in the *C. xanthorrhiza* such as flavonoids also have hepatoprotective activity.

Criteria of Pearson correlation analysis: 0: No correlation, 0.00–0.25: A very weak correlation, 0.25–0.50: Enough correlation, 0.50–0.75: Strong correlation, 0.75–0.99: A very strong correlation, 1: Perfect correlation.[28]

DISCUSSION

This study presents a considerable hepatoprotective activity of *C. xanthorrhiza* using some samples such as FRD, DRD, essential oils, and curcumin on PCT-induced liver damage in rats and correlated their chemical compounds with pharmacological activity. Overdosage of the PCT will result in accumulation of NAPQI, which will bind to glutathione (GSH) to form conjugates that will lead to the oxidation and conversion of GSH to glutathione disulfide, resulting in the reduced level of blood and liver GSH.[22] These events initiate necrotic cell death and abnormalities of the liver.[7,8] Necrotic cell death releases the biochemical markers such as SGPT and SGOT into the circulation.[29]

Indonesian medicinal plant that prospect for the treatment of liver diseases is *C. xanthorrhiza*. It has been used as food and medicinal purposes to treat hepatitis, liver disorders, stomach diseases, rheumatism, skin inflammation, anticariogenic, antifungal, anti-Malassezia, antimycotic, and antimicrobial.[30,31] Empirically, *C. xanthorrhiza* is used for liver disease treatment using FRD at a dose of 25 g daily.[15]

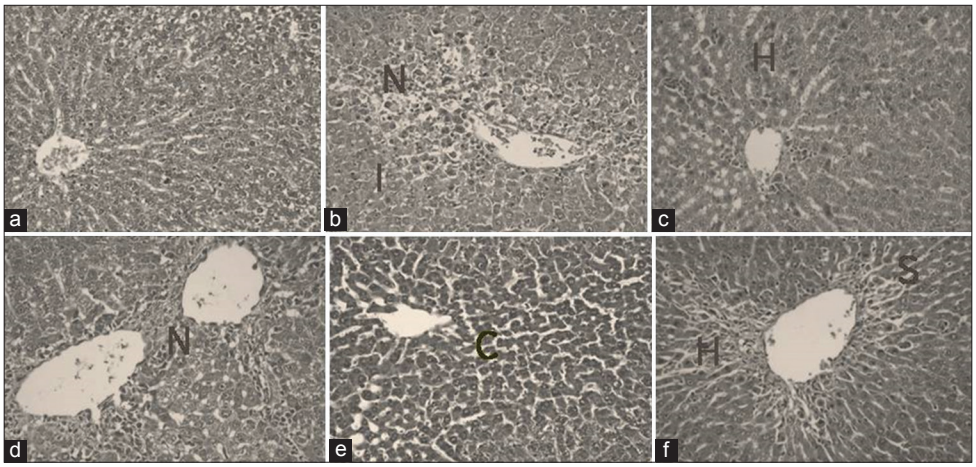

Figure 2: (a) Normal, (b) paracetamol (3 g/kg BW), (c) fresh rhizome decoction (6.75 g/kgBW), (d) dried rhizome decoction (4.05 g/kg BW), (e) essential oils, (f) curcumin, I: Inflammation, N: Necrosis, C: Congestion, H: Hemorrhage, S: Steatosis

Table 5: Pearson analysis of curcumin and essential oils contents against hepatoprotective effect

No	Hepatoprotection effect	Essential oils	Curcumin
		Pearson	Pearson
1	SGPT protection effect	0.481	0.256
2	SGOT protection effect	0.595	0.463

SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvate transaminase,

The rhizomes of *C. xanthorrhiza* contain essential oils, xanthorrhizol, curcuminoids, flavonoids, ar-curcumene, terpenoids, phenol, and saponin.[20,32] Essential oils are major compounds of *C. xanthorrhiza* which contains many chemical compounds such as xanthorrhizol, diepi-α-cedrene, α-curcumene, champor, and germacrene B.[33] Currently, no studies have reported hepatoprotective activity of essential oils, but there is a report that xanthorrhizol has hepatoprotective activity. Xanthorrhizol is an active compound of *C. xanthorrhiza* responsible for the hepatoprotective activity. Xanthorrhizol has better hepatoprotective activity than curcumin. Mechanisms of the hepatoprotective effect of xanthorrhizol may be related to its capability to regulate DNA-binding of transcription factors, nuclear factors kappa B (NF-αB) and AP-1.[34]

Curcumin is a major compound and also plays a role in the hepatoprotective activity. Curcumin is able to inhibit GST and cytochrome P450 activities.[35,36] Mechanisms of the preventive effect of curcumin may be related to its inhibition of lipid peroxidation and oxidation stress. Curcumin restored Bcl-2/Bax ratio, thus reducing the PCT-induced hepatocyte apoptosis. Bcl-2 protein is commonly recognized as an anti-apoptotic factor, and it inhibits cell apoptosis by preventing mitochondrial membrane depolarization. As a member of the Bcl-2 family, Bax inactivates Bcl-2 by interacting with it to form a heterodimer. Curcumin can downregulated the mRNA expression of Bax and upregulated the mRNA expression of Bcl-2. Curcumin also exerts a potent anti-apoptotic effect through inhibition of TGF-β as an inducer of caspase-3-mediated apoptosis.[37]

Flavonoids are minor compounds of *C. xanthorrhiza,* Flavonoids have correlation with antioxidant and hepatoprotective activity.[38] Flavonoids are able to induce antioxidant defense through activation of Nrf2 and reduction of inflammation through inhibition of NF-αB.[39] All chemical compounds of *C. xanthorrhiza* such as essential oils, xanthorrhizol, curcumin, and flavonoids have contributed to hepatoprotective activity.

CONCLUSIONS

It is evident from the data obtained from this study that all treatment groups of *C. xanthorrhiza* samples have a hepatoprotective effect on PCT-induced liver damage in rats. Among all the samples, FRD at a dose of 6.75 g/kg BW showed better activity compared to the other samples. The percentage of the protection of each group was different because of the distinction of phytochemical profiles such as curcumin level, essential oils level, variation chemical compounds of essential oils, and flavonoids. Essential oils have strong correlation than curcumin on PCT-induced liver damage in rats, and all chemical compounds have a hepatoprotective activity.

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